Characterization of BBa_F2620, an Engineered Cell-Cell Communication Device

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Abstract

Current research in synthetic biology aims to enable the design and construction of biological systems in the manner that we now engineer electrical, mechanical, and other man-made constructs. To enable rational design and construction of synthetic biological systems we need to be able to predict the behavior of a system from the characteristics of the components that comprise it. Thus, designing and using a methodology for characterizing the performance of biological parts and devices is central to the future success of biological engineering. While a wide range of devices and systems has been described, there has been insufficient characterization of those devices and systems. This work presents the first attempt at comprehensive characterization of a standard biological part. We engineered a cell-cell communication device, BBa_F2620, based on parts from the quorum sensing system of *Vibrio fischeri*. BBa_F2620 is a receiver device that responds to the concentration of a small signaling molecule AHL (N-(-Ketocaproyl)-DL-homoserine lactone) in the media by modulating the transcription rate that is output from the device. To begin to systematically characterize BBa_F2620, we defined characteristics important for performance based design and composability. To measure those characteristics we connected the receiver upstream of BBa_E0240, a fluorescent protein-based reporter device, and measured the following properties: device INPUT/OUTPUT transfer function (including switch point, latency, and variation across clonal colonies), input signal specificity, and device stability (both genetic and performance), using multiwell fluorimetry and flow cytometry. Using these characteristics we populated a first-generation datasheet that describes the use and operation of BBa_F2620. Families of devices characterized in the manner described here will accelerate the development of larger-scale systems.

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1. INTRODUCTION

Advances in the field of synthetic biology are making it feasible to engineer biological systems. Biological systems offer properties and functions not found in traditional engineering systems and have broad applications in medicine, biology and engineering. To enable the design of multi-device assemblies, it is necessary to be able to predict system function from the constituent devices of the system. Such prediction requires that the behavior of the constituent devices be well specified and documented. Furthermore, this characterization must be done in a consistent manner such that the characterized devices can be composed reliably, systematized, catalogued and compared across research institutions. While a wide range of devices and systems has been described [1–4], there has been insufficient characterization of them to allow the rapid, reliable assembly of more complex systems.

We identified a set of characteristics that specify the performance of a simple biological device and measured these characteristics for a cell-cell signaling receiver device, F2620. A fluorescent reporter device was used to measure the output of the receiver device. By applying a similar approach to other devices, a library of well-characterized and composable devices could be generated.

Cell-cell communication allows individual cells to coordinate their behavior with the rest of the population and as such is a powerful technology for engineering complex biological systems. F2620 is a receiver device that responds to the concentration of a small signaling molecule (an acyl-homoserine lactone or AHL molecule) in the extracellular media by modulating the transcription rate from a promoter. Hence, we define the input to the device to be extracellular concentration of AHL and the output to be transcription rate. The device is based on elements of the quorum sensing system of *Vibrio fischeri*. The quorum sensing system includes an enzyme, LuxI, that synthesizes an AHL molecule (N-(b-ketocaproyl)homoserine Lactone). LuxR is a transcriptional activator protein that is active when bound to AHL. When active, it binds to the Lux box and recruits RNA polymerase to the operator region [5-8]. F2620 consist of six standard parts (Figure 1). A tet repressible promoter (R0040), followed by ribosome binding site (B0034), drives production of LuxR from the luxR coding region (C0062). Transcription from TetR promoter is terminated by two transcription terminators (B0010, B0012) to ensure 100% termination. The sixth part is a Lux_pR promoter (R0062) that contains a LuxR binding site. This promoter is the right-

most part of the *V. fischeri* Lux operator. To measure the output from F2620 we connected a GFP reporter device E0240 downstream of F2620 (not shown on Fig. 1).

The transfer function relating device input to output is the primary characteristic for any device. For the receiver device, we measured the input by adding a known concentration of AHL to the culture media. We measured the output by calculating the rate of GFP accumulation per optical density (OD). We derived certain parameters that capture the key characteristics of the transfer curve - Hi/Lo input and output values, switch point, performance variability between genetically identical clones, input signal specificity, latency, and device stability (genetic and performance). The GFP reporter device was chosen because it allowed reliable, high time-resolution measurements to be made via multiwell fluorimetry and flow cytometry.

2. RESULTS ANALYSIS AND DISCUSSION

The maximum output level, Hi value, was determined to be 247 $\text{GFPs}^{-1}/\text{OD} \pm 23\%$ and was observed above an input of 10E-7M AHL. The device was considered to be off (Lo value) when GFP accumulation rate was below 5% of the maximum output, which occurred below 10E-10M AHL. The switch point for the device, the input concentration at which output is at 50% of the maximum, is 10E-9M AHL.

We measured the performance variation between genetically identical colonies taken from long-term storage using multiwell fluorimetry. The average performance of cultures grown from 6 colonies is 312 $\text{GFPs}^{-1}/\text{OD}$. The coefficient of variation in the Hi value among the 6 colonies is 8.3% and is evenly distributed above and below the mean. Other tested AHL concentrations above the switch point show a coefficient of variation below 25% (see Fig. 1).

We sought to quantify the ability of the device to distinguish between its cognate inducer AHL (N-(-Ketocaproyl)-DL-homoserine lactone) and a range of chemically similar inducers with varying length side chain (N-Hexanoyl-DL-homoserine lactone, N-Butyryl-DL-homoserine lactone, N-Heptanoyl-DL-homoserine lactoneN-Octanoyl-DL-homoserine lactoneN-Decanoyl-DL-homoserine lactone , N-Dodecanoyl-DL-homoserine lactone , N-Tetradecanoyl-DL-homoserine lactone). Fig. 1 shows transfer curves obtained using the different AHL molecules as inputs. The maximal output of the device (Hi level) shows strong

dependence on the specific inducer. The cognate AHL produces the highest output level of 261 $GFPs^{-1}/OD$. A similar inducer lacking a carbonyl group and having chain length intact or extended to 7, 8 or 10 carbon atoms shows response decreased by less than 16% of cognate response at the highest tested AHL concentration (1E-5M). When the AHL molecules have their side chains extended further to 12 or 14 carbon atoms or shortened to 4 carbon atoms, activation is visible, but its maximum level is less than 18% of the cognate inducer at maximum output. It can be seen that the switch point for each of AHL variants is constant at 10E-9M.

Latency is defined as the time delay between the change in input concentration and the output level reaching 95% of its final value. These values were obtained by measuring the rate of GFP accumulation per second per OD at a high induction level in one minute intervals using multiwell fluorimeter until a constant accumulation rate characteristic for steady state receiver operation was obtained (data not shown). The rate reaches Hi value defined as 95% of a steady state plateau of 215 $GFPs^{-1}/OD$ after 7min. Subsequently, transcription was stopped using Rifampicin and the output of the device decreased to reach a Lo value (defined as 5% of steady state level) after 86 min. This implies off/on latency of 7min and on/off latency of 86 min for the receiver-reporter construct.

Device stability was investigated under different operating conditions by propagating the culture through 92 doublings over the course of 5 days to asses how fast evolutionary pressure will induce mutations in the device in order to relief additional load imposed by GFP production. Performance under low input conditions, assayed using multi well fluorimetry, shows slight variations in GFP accumulation rate over the course of the experiment (coefficient of variation being 12%). The performance of the device working under high input conditions shows similar variations during first three days of the experiment; however, in the fourth day, after 74 doublings, the high output level dropped to approximately 1% of the original level and on the fifth day the high output had fallen further to less than 0.9% (data not shown). In order to gain more insight into the mechanism of failure, single-cell performance was investigated using flow cytometry and showed that the population of cells split on day 4 after 74 doublings into two groups: a more populous one, which was not-activated (82%) and a less populated one (18%), which still retained fluorescence (Figure 1, bottom). On the last day only a few visibly fluorescent cells remained. The DNA sequence of the receiver and reporter device remained unchanged over the course of the experiment when

the device was operated with low input. This suggests the evolutionary stability of composed receiver-reporter device to be around 74 doublings and the receiver device alone to be over 92 doublings. When operated with a high input, approximately 82% of cells acquired a mutation in the receiver sequence that completely prevented GFP production. This is due to a mutation leading to complete removal of the GFP reporter device. This mutation is most likely due to recombination between homologues transcription terminator regions of the receiver and reporter device. Experiments to validate this hypothesis are pending.

3. CONCLUSIONS

This work presents the first attempt to comprehensively characterize a standard biological part, which has a multi-fold importance. In the process of characterizing BB_F2620 we laid the foundations of an engineering methodology for the future characterization of biological parts and populated a first-generation datasheet that describes the use and operation of BB_F2620.

BBa_F2620

3OC$_6$AHL → PoPs

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Last Update: April 2, 2006

Description and Usage:

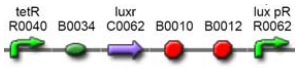
***Device input* is 3OC$_6$HSL. *Device output* is GFP/s·OD** (related to polymerases per second, **PoPS,** produced from activated LuxpR receiver) produced at a LuxR-regulated operator A transcription factor [LuxR] that is active in the presence of cell-cell signaling molecule [3OC$_6$AHL] is constitutively expressed from an operator [TetR].Full GFP/s·OD output at high 3OC$_6$ AHL levels and low plasmid copy [e.g., pSB3K3] results in a reduced cell growth rate. If used in a cell containing TetR then a second input signal [aTc] can be used to produce a logical *AND* function.

Characteristics

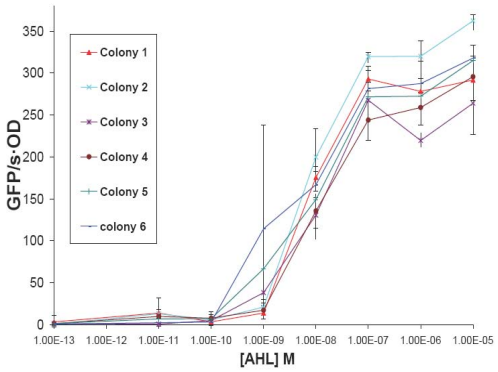
Full Output: 247 GFP/s·OD ± 23%
Full Output Variability Coefficient: **8.3%**
Switch Point: **10 nM** 3OC$_6$AHL, exogenous
LH Latency: **7** minutes
HL Latency: **86** minutes

Key Components

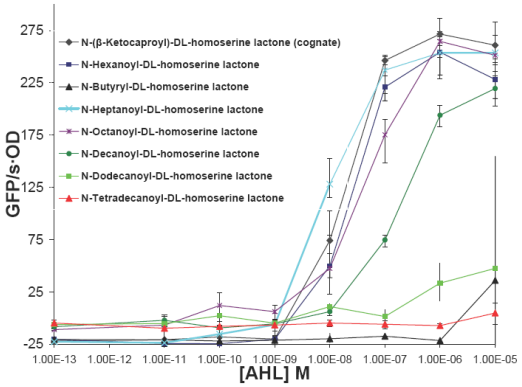
BBa_R0040: TetR-regulated operator
BBa_C0062: luxR ORF
BBa_R0062: LuxR-regulated operator



Transfer Function Variability:

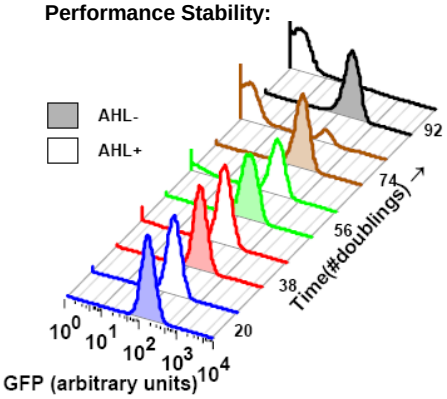


Specificity:



Signaling Devices

Performance Stability:



Full Induction: device non-functional after 74 doublings

No induction: device functional for over 100 doublings

Compatibility

Device has been shown to work in *MC4100*, *MG1655*, and *DH-5α*.

Device has been shown to with on *pSB3K3* and *pSB1A2*.

Device has been shown to work with *E0430* and *E0434*.

Crosstalk with systems containing *TetR, some molecules of AHL moiety*.

*Device output measured indirectly via fluorescence from *BBa_E0430*, [ ] = geometric mean, arbitrary units. Host cell *MG1655*, device carried on *pSB3K3*, 5ml batch flask, supplemented M9 media, FACScan cytometer [see *MIT SBWG FACS protocol*].

FIG. 1: Comprehensive datasheet for BBa_2620, cell-cell communication device, produced as a result of characterization procedure. Important characteristics were defined to be: device INPUT/OUTPUT transfer function, input signal specificity, and device stability (both genetic and performance). Transfer function variability describes how the output rate of GFP/sOD varies with the input concentration of cognate AHL for six different clonal colonies taken from the same long-term storage. Specificity transfer functions describes how the output rate of GFP/sOD varies with the input concentration of cognate AHL and its various derivatives Transfer curves have been measured using Wallac Victor3 multi-well fluorimeter. Performance stability FACS histogram representing the number of cells expressing GFP with a given intensity (arbitrary units) after certain number of doublings. Cultures propagated without AHL (shaded) maintain uniform GFP expression over the course of experiment while those that were propagated under full load condition (no shading) unable to express GFP after 74 doublings.

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4. ADDENDUM. MY SPECIFIC ROLE IN THE RESEARCH PROJECT

I joined Prof. Endys lab in the summer of 2005 and began characterization of BB_F2620, an engineered cell-cell communication device, with Prof. Drew Endy and PhD candidate, Barry Canton. When I joined the lab, discussion about the important characteristics of the device was already underway and there existed a proof of concept that the receiver transfer function could be measured using a fluorescent reporter device. I first assembled the receiver device with an improved reporter device using standard molecular cloning techniques and helped define a methodology for characterization of biological devices. Next, I designed the experimental protocols to characterize the receiver device. Finally, I performed the experimental work with the help and guidance of Barry, Drew and other lab members. Experiments included multiwell fluorimeter calibration, population assays using flow cytometry and multiwell fluorimetry as well as genetic assays done by extracting plasmid DNA and sequencing it[1]. I was also responsible for analyzing vast amounts of numerical data yielded by those experiments. In order to facilitate rapid and robust data processing I developed VBA scripts that processed and analyzed the data.

I have presented results of the project on numerous group meetings including meeting on Error Detection & Correction in Replicating Machines (Boston, Nov.2005). Recently, I was able to present my findings at the eleventh annual meeting of institute of biological engineers (Tucson, March 2006), where they were met with great interest. Currently, I am drafting a research manuscript for submission to a peer-reviewed journal.

[1] Exact experimental protocols can be found at http://sblab8.mit.edu/ Barry/mediawiki/index.php/Main_Page